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Novel opsin gene variation in large-bodied, diurnal lemurs

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Abstract

Some primate populations include both trichromatic and dichromatic (red-green colour blind) individuals due to allelic variation at the X-linked opsin locus. This polymorphic trichromacy is well-described in day-active New World monkeys. Less is known about colour vision in Malagasy lemurs, but, unlike New World monkeys, only some day-active lemurs are polymorphic, while others are dichromatic. The evolutionary pressures underlying these differences in lemurs are unknown, but aspects of species ecology, including variation in activity pattern, are hypothesized to play a role. Limited data on X-linked opsin variation in lemurs make such hypotheses difficult to evaluate. We provide the first detailed examination of X-linked opsin variation across a lemur clade (Indriidae). We sequenced the X-linked opsin in the most strictly diurnal and largest extant lemur, *Indri indri*, and nine species of smaller, generally diurnal indriids (*Propithecus*). Whereas nocturnal *Avahi* (sister taxon to *Propithecus*) lacks a polymorphism, at least eight species of diurnal indriids have two or more X-linked opsin alleles. Four rainforest-living taxa - *I. indri* and the three largest *Propithecus* species - have alleles not previously documented in lemurs. Moreover, we identified at least three opsin alleles in *Indri* with peak spectral sensitivities similar to some New World monkeys.

Keywords: *Indri*, colour vision, primate, *Propithecus*, sensory ecology

Introduction

Many primate species exhibit "polymorphic trichromacy" through allelic variation of a single X-linked (medium-to-long wavelength sensitive) opsin gene [1]. As a result, all males and homozygous females are inferred to be red-green colour-blind (dichromatic), while heterozygous females are inferred to be trichromatic [1]. This variation is thought to be maintained, in part, by adaptive advantages conferred to trichromats. Many hypotheses highlight the potential utility of trichromacy for foraging on "reddish" food (fruit, young leaves), particularly during the day when light levels are more conducive to colour vision (reviewed in [2,3]).

Polymorphic trichromacy is both widespread and highly variable (>2 opsin alleles) among most diurnal New World monkeys and is found in some day-active Malagasy lemurs (2 alleles) [2-8]. Yet other day-active lemurs appear to have a single opsin allele and are strictly dichromatic [9-11]. There is some behavioural evidence linking differences in opsin gene variation to differences in colour vision phenotypes among lemurs [8,12], and it has been suggested that this variation might be influenced by ecological differences, including the degree of diurnal behavior [10]. This hypothesis has been difficult to evaluate, however, because X-linked opsin data are only available for a few lemur taxa [6-11]. To help fill this gap, we provide the first comprehensive examination of X-linked opsin variation across members of the lemur family Indriidae. Indriid species share some ecological traits (e.g. folivory), but they vary in body size and activity pattern. The largest extant lemur, *Indri indri*, is the most strictly diurnal lemur species [13,14]. Related to *Indri* are the sister taxa *Avahi* (nocturnal) and *Propithecus* (diurnal, with some activity at dawn/dusk) [13-16].

Methods

Amino acid changes at three sites (180: exon 3; 277, 285: exon 5) in the X-linked opsin gene(s) are primarily responsible for spectral differentiation of medium/long wavelength sensitive opsins in primates [17,18], and at least in some primate opsin alleles (found in New World atelines), changes at site 294 can also have an effect [19] Although all three primary sites (180, 277, 285) are variable in polymorphic New World monkeys, variation in lemurs has only been documented in site 285 leading to two alleles: alanine 180, tyrosine 277, alanine 285 (AYA), and alanine 180, tyrosine 277, threonine 285 (AYT), which encode opsins with peak spectral sensitivities (λ_{max}) ~543 nm ("M") and ~558 nm ("L"), respectively [2-8]. We sequenced exons 3 and 5 to capture the three key functional sites in the X-linked opsin gene of indriids (supplementary material).

We genotyped the X-linked opsin for 151 wild individuals representing all nine species of *Propithecus* and the single species of *Indri* (*I. indri*) across 15 sites in Madagascar (Figures 1&2; supplementary material). We pooled populations to characterize opsin allele variation for each species (for *P. perrieri* and *P. candidus* we had data for a single individual). Data for wild *Avahi* (nine species) were previously published [20].

Results

We identified substantial variation in the amino acid composition of opsin alleles (Figure 2; see [21] for sequence alignments). Eight species of diurnal indriids had two or more alleles, and heterozygotes were only found among females, indicating they have polymorphic trichromacy. For five *Propithecus* species (*P. coquereli*, *P. coronatus*, *P. deckenii*, *P. tattersalli*, and *P. verreauxi*), we identified the two alleles previously found in lemurs [6] based on variation at site 285 (Figure 2): AYA (λ_{max}~543 nm) and AYT (λ_{max}~558 nm). All spectral sensitivities are

based on the estimated effects of differences in the three spectral tuning sites [5,6]. The AYT allele was also found in the single *P. perrieri* individual.

Two *Propithecus* species – *P. diadema* and *P. edwardsi* – have variation at functional sites 277 and 285 in exon 5. In both species, an allele with three-site composition AFA is found, with λ_{max} ~535 nm [5]. The other X-linked opsin is shared with other *Propithecus* (AYT, λ_{max}~558 nm). We also identified the AFA allele in the single *P. candidus* individual.

Indri exhibits the most allelic variation observed to date in strepsirrhine primates. Unlike previous studies, we identified amino acid variation at all three functional sites and verified at least three alleles. We confirmed two novel opsins in lemurs. One allele has a three-site composition of serine, phenylalanine, and threonine (SFT): λ_{max}~555 nm [5]. (Note: site 294 did not vary [19]). The other is composed of serine, tyrosine, and threonine (SYT): λ_{max}~562 nm [5].

Three females were heterozygous at all three functional sites, identifying two potential allelic combinations based on haplotype phasing of exon 5: AFA/SYT and SFA/AYT. In other cases, haplotypes could be inferred from homo/hemizygotes. Given the presence of the SYT allele in *Indri*, and AFA allele in three species of *Propithecus*, AFA/SYT is the most likely allelic combination, but we cannot rule out the potential presence of SFA and AYT alleles. Accordingly, we identified at least three and up to five alleles in *Indri*.

Discussion

Our results reveal that the X-linked opsin gene in lemurs is more variable than previously thought. Prior studies identified two opsin alleles based on spectral-shifting variation at a single site (285) [6-10]; we uncovered variation at additional sites (180, 277), leading to the identification of at least three alleles not previously described in lemurs. We documented polymorphic trichromacy in eight species of diurnal indriids, including six species for which

115 opsin allele variation was previously unknown [6,8]. *Propithecus perrieri* and *P. candidus* might
116 also prove polymorphic with the genotyping of additional individuals. In contrast, the closely-
117 related nocturnal genus *Avahi* is dichromatic with a single X-linked opsin [20]. Notably, the
118 largest-bodied, most strictly diurnal lemur, *Indri indri*, exhibits the most variation. It is the only
119 lemur currently identified to have variation at site 180, and the only species with three alleles
120 confirmed.

121 The three 'novel' alleles we identified in *Indri* and some species of *Propithecus* (AFA,
122 SFT, and SYT) have not been described among strepsirrhines, but they have been identified in
123 diurnal haplorhines [2-5,22,23]. AFA and SYT occur among routinely trichromatic catarrhines
124 and platyrrhine howling monkeys as two separate X-linked opsin gene variants, and are common
125 allelic variants among polymorphic platyrrhines [2-5,22,23]. SFT is more rare, having only been
126 documented in New World Atelids, *Cacajao*, and *Cebus* [5,22,23]. Studies modeling the colour
127 vision of haplorhines suggest that their photopigments are optimized for detecting food items in a
128 rainforest environment [24,25], and interestingly, the 'novel' spectral sensitivities we observed in
129 lemurs are limited to the largest-bodied, diurnal species living in the eastern rainforests of
130 Madagascar. All other diurnal species that exhibit the two alleles previously described in lemurs
131 occur in dry forest habitats [16]. Thus, although not explicitly tested in this study, opsin gene
132 variation in lemurs might be influenced by various ecological factors, including activity pattern,
133 diet, habitat, and predation pressure [8,10,26,27].

134 Overall, our study has revealed greater variation in lemur opsin genes, and the novel
135 variation we identified is so far limited to the largest-bodied, diurnal indriids. Given that most
136 large-bodied, diurnal lemurs have recently gone extinct (last 2,000 years) [28], the opsin
137 variation observed today could underrepresent the full range of variation in lemurs. New

paleogenomic techniques may reveal additional variation in recently-extinct subfossil lemurs [29], further expanding our understanding of opsin gene diversity in this lineage.

Ethics statement

All procedures were approved by and adhered to institutional and national guidelines. Procedures were approved by the Institutional Animal Care and Use Committees (IACUC) of Stony Brook University (IACUC# 2003-1169, 2005-2008-1449) and Omaha's Henry Doorly Zoo and Aquarium (IACUC# 97-100, 12-101).

Data accessibility

Sequence data are available through Dryad (Provisional DOI: doi:10.5061/dryad.q1d1s).

Authors' contributions

RLJ, TSM, NIM, BJB, ANS analyzed data. ALB, EELJ, TLM, MTI, RRL, JP, MM, RL, RC, MTRH, PMK, PCW acquired samples/data. RLJ, BJB, NIM drafted the manuscript. All authors revised the manuscript critically, approved the final version of the manuscript, and agree to be accountable for its content.

Competing interests

We have no competing interests.

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Figure 1

Ten Indriidae species were sampled from sites across Madagascar (stars). Data from *Avahi* sites (circles) are from [20]. *indicates sites that include *Avahi* data.

Figure 2

X-linked opsin amino acid variants at three key functional sites for each genotype. Peak spectral sensitivities estimated based on spectral tuning sites following [5,6]. The number of females (F) and males (M) exhibiting each genotype is provided. Phylogeny from [30]. Body size data from [16]. Lemur illustrations copyright Stephen D. Nash, used with permission.